



***Chemical Analysis and Testing Task
Laboratory Analytical
Procedure***

LAP-010

Procedure Title:	Standard Method for the Determination of Extractives in Biomass
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Standard Method for the Determination of Extractives In Biomass

Laboratory Analytical Procedure #010

1. Introduction

- 1.1 With many types of biomass feedstocks it is necessary that the extractives be removed from the sample prior to analysis to prevent interference with the analytical procedure. Historically, ethanol-benzene has been used to extract waxes, fats, some resins, and portions of wood gums. Subsequent hot water extractions were then used to remove tannins, gums, sugars, starches, and coloring matter. Soxhlet extraction with 95% ethanol has been found to be an effective, non-toxic alternative to extractions employing benzene.
- 1.2 This procedure has been accepted by ASTM as an ASTM Standard Test Method for the determination of extractives in biomass feedstocks.

2. Scope

- 2.1 This test method covers the determination of ethanol soluble extractives, expressed as the percentage of the oven-dried biomass, of hard and soft woods, herbaceous materials, agricultural residues, and wastepaper.
- 2.2 All analyses shall be performed in accordance with the Ethanol Project Quality Assurance Plan (QAP).

3. References

- 3.1 ASTM D1105-84, "Method for Preparation of Extractive-Free Wood." In *1993 Annual Book of ASTM Standards, Volume 04.09*. Philadelphia, PA: American Society for Testing and Materials.
- 3.2 Moore, W., and D. Johnson. 1967. *Procedures for the Chemical Analysis of Wood and Wood Products*. Madison, WI: U.S. Forest Products Laboratory, U.S. Department of Agriculture.
- 3.3 NREL CAT Task Laboratory Analytical Procedure #001, "Determination of Total Solids and Moisture in Biomass."
- 3.4 NREL Chemical Technologies Research Branch Procedure #001c, "Determination of Extractives Content."
- 3.5 TAPPI Test Method T204, "Solvent Extractives of Wood and Pulp." In *Tappi Test Methods*. Atlanta, GA: Technical Association of the Pulp and Paper Industry.

4. Significance and Use

- 4.1 Extractives, as defined by this procedure, are the fraction of a biomass sample soluble in ethanol and that are left as a residue following exhaustive Soxhlet extraction. Extractives include non-structural components of biomass samples which potentially could interfere with the analysis of the biomass sample, and as such must be removed prior to compositional analysis.
- 4.2 This method gives results comparable to ASTM Method D1105-84.

5. Apparatus

- 5.1 *Soxhlet extraction apparatus* - A glass Soxhlet extraction apparatus of suitable size (100 mL) for containing the sample and a 250 mL collection flask is required for the conventional Soxhlet procedure. An automated extraction apparatus (Brinkmann Buchi B-810 or equivalent) with circulating oil bath and associated glassware is required for the automated Soxhlet procedure.
- 5.2 *Alundum extraction thimbles* - Medium porosity (10 - 15 μm pore), sized to fit the Soxhlet extractor.
- 5.3 *Analytical balance* - Sensitive to 0.1 mg.
- 5.4 *Rotary evaporator with vacuum and water bath* - Rotary evaporator equipped with a "bump" trap, condenser, receiving vessel, and vacuum source sufficient to pull a vacuum of less than 150 torr.
- 5.5 *Vacuum oven or drying oven* - Vacuum oven should be controllable to a temperature of $40 \pm 1^\circ\text{C}$ and vacuum of between 75 to 100 torr. If drying oven is used in place of the vacuum oven, the drying oven must be able to maintain $45 \pm 2^\circ\text{C}$.

6. Reagents and Materials

- 6.1 Ethyl alcohol, 95% in water (190 proof), USP grade.
- 6.2 Boiling chips.
- 6.3 Glass wool.
- 6.4 Buchner funnel.
- 6.5 Desiccator.

7. ES&H Considerations and Hazards

- 7.1 Ethanol is a flammable reagent.
- 7.2 Follow all applicable NREL Laboratory Specific Hygiene Plan guidelines.

8. Sampling and Test Specimens

- 8.1 The test specimen shall consist of approximately 10 grams of milled sample obtained in such a manner as to ensure that it is representative of the entire lot of material being tested.
- 8.2 If the sample requires milling prior to extraction, pass the sample through a 40 mesh screen (a laboratory scale Wiley mill is recommended for this milling step). Wet samples will require air drying prior to milling.

9. Procedure

- 9.1 Dry the Soxhlet extraction thimble at 105°C to constant weight. Remove, cool to room temperature in a desiccator, and weigh to the nearest 0.1 mg.
- 9.2 Carefully add the sample to the extraction thimble. Do not overfill the thimble, leave at least a 1 cm gap between the sample and the top of the thimble. Weigh the filled thimble to the nearest 0.1 mg. Place a plug of glass wool on top of the sample to prevent sample loss during the extraction.

Note: Samples for total solids determination (following Laboratory Analytical Procedure #001, Determination of Total Solids and Moisture in Biomass) must be weighed out at the same time as the samples for the extractives determination. If this determination is done at a later time, an error in the calculation of the amount of extractives will be introduced, since the moisture content of a biomass sample can change rapidly when exposed to air.

- 9.3 Place several boiling chips into a clean, dry receiving flask or beaker. Weigh the container, with chips, to the nearest 0.1 mg and record as the tare weight of the container.
- 9.4 For a conventional Soxhlet extraction (this procedure was reproduced from the Chemical Technologies Research Branch Procedure #001c, Determination of Extractives Content):
 - 9.4.1 Assemble the Soxhlet apparatus using at least 160 mL of 95% ethanol. Insert the thimble and heat at reflux for 24 hours. Periodically check the reflux rate and adjust the heating rate to give four to five solvent exchanges per hour in

the Soxhlet thimble. Approximately 100-120 solvent exchanges are required during the 24 hour period.

- 9.4.2 When the extraction time is complete, remove the thimble and carefully transfer the sample to a Buchner funnel. Remove any residual solvent by vacuum filtration and wash the sample thoroughly with 95% ethanol, collecting all of the filtrate. Allow the biomass to air dry in the Buchner funnel while it is still attached to the vacuum system.
- 9.4.3 Combine the filtrate from the previous step and any solvent from the upper section of the Soxhlet apparatus with the solvent in the 250 mL flask. Place the flask on the rotary evaporator and remove the solvent under vacuum. Use a water bath temperature of $45 \pm 5^{\circ}\text{C}$ to heat the flask during evaporation.
- 9.4.4 After all of the visible solvent is removed by the rotary evaporator, place the flask in a vacuum oven (75-100 torr) at $40 \pm 1^{\circ}\text{C}$ for 24 ± 1 hour. Remove the flask at this time and allow to cool to room temperature in a desiccator. Weigh the flask and record this total weight to the nearest 0.1 mg.

9.5 For an automated Soxhlet extraction:

- 9.5.1 Turn on the circulating oil bath, and set to 170°C .
- 9.5.2 Add approximately 100 mL of 95% ethanol to the receiving beaker. Place the thimble containing the sample inside the extractor tube. Finish assembling the automated Soxhlet extractor as directed in the instrument manual.
- 9.5.3 Begin the extraction, verifying that the reflux rate is ten to twelve solvent exchanges per hour. Reflux for eight hours, giving a total of 80 to 100 solvent exchanges.

Note: An overnight extraction may be used. Select a circulating oil bath temperature (and, indirectly, a solvent exchange rate) that will produce approximately 100 solvent exchanges during the extraction period. Verify that the apparatus is free of leaks so that there is no loss of solvent during the period of unattended operation.

- 9.5.4 At the end of the extraction, remove the thimble and transfer to a Buchner funnel. Remove any residual solvent by vacuum filtration and wash the sample thoroughly with 95% ethanol, collecting all of the filtrate. Allow the biomass to air dry in the Buchner funnel while it is still attached to the vacuum system.

- 9.5.5 Combine the filtrate, the solvent from the extractor tube, and the solvent in the beaker. Evaporate to dryness using a rotary evaporator, as described in the conventional Soxhlet extraction section, or alternatively by using the automated extraction apparatus, as described in the next two steps.
- 9.5.6 Leaving the beaker in place on the heating block, decrease the temperature of the circulating oil bath to 100°C. Evaporate away the solvent until only about 10 mL remains.
- 9.5.7 Place the beaker in a drying oven set (45°C) or vacuum oven (75-100 torr and 40°C) for 24 ± 1 hour. Remove the beaker, cool to room temperature in a desiccator, and weigh to the nearest 0.1 mg.

10. Calculations

- 10.1 Calculate the oven dry weight of the sample, using the average total solids content as determined by the Laboratory Analytical Procedure #001, Determination of Total Solids and Moisture in Biomass.

$$ODW = \frac{(Weight, \text{thimble plus sample} - Weight, \text{thimble}) \times \% \text{Total solids}}{100}$$

$$\% \text{ Extractives} = \frac{Weight \text{ container plus residue} - Tare \text{ wt. container}}{ODW} \times 100$$

- 10.2 Calculate the amount of extractives in the sample, on a percent dry weight basis.

11. Report

- 11.1 Report the average percent extractives in the sample on an 105°C dried weight basis, along with the standard deviation and the relative percent difference.

12. Precision and Bias

- 12.1 Data obtained by replicate testing of a hybrid poplar sample in one laboratory gave a standard deviation in extractive content of 0.15% and a CV% of 7.6%. Replicate testing of a National Institute of Standards and Technology (NIST) #8494 wheat straw gave a standard deviation of 0.20% and a CV% of 1.6% and NIST #8493 Pinus radiata gave a standard deviation of 0.20% and a CV% of 8.0%.
- 12.2 Prolonged heating of the extractive residue may bias the reported results low because of evaporation of semivolatile constituents. Insufficient heating or using inadequate vacuum can bias the results high because of incomplete removal of the ethanol solvent.

13 Quality Control

- 13.1 *Reported significant figures:* All results shall be reported as a percentage with two decimal places.
- 13.2 *Replicates:* All samples and method verification standards shall be analyzed in duplicate.
- 13.3 *Blank:* It is recommended that a solvent blank be run with every batch of samples.
- 13.4 *Relative percent difference criteria:* The %RPD must be less than 10%. If the %RPD is too large, the sample will be rerun.
- 13.5 *Method verification standard:* It is recommended that a method verification standard be run with every batch. This standard is a material of known extractives content that is run in parallel with the samples to track the reproducibility of the analysis.
- 13.6 *Calibration verification standard:* Not applicable.
- 13.7 *Sample size:* The sample, added to an extraction thimble, shall be a minimum of three grams or the results will be flagged as having compromised precision.
- 13.8 *Sample storage:* Store the extracted sample in the refrigerator until needed for further analysis.
- 13.9 *Standard storage:* Not applicable.
- 13.10 *Standard preparation:* Not applicable.
- 13.11 *Definition of a batch:* Any number of samples which are analyzed together and recorded together. The maximum size of a batch is limited by the equipment constraints. A batch cannot be larger than what is practical with the available equipment.
- 13.12 *Control charts:* All method verification standards shall be control charted.